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011479410 **Image available**
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Screening for apoptosis inducers such as antibodies, for use as anticancer agents - by use of cells expressing integrin -associated protein as screen
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Inventor: FUKUSHIMA N
Number of Countries: 076 Number of Patents: 004
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WO 9732601	A1	19970912	WO 97JP702	A	19970306	A61K-039/395	199742 B
AU 9722325	A	19970922	AU 9722325	A	19970306	A61K-039/395	199804
JP 9295999	A	19971118	JP 9767499	A	19970306	C07K-016/28	199805
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Abstract (Basic): WO 9732601 A

A screen for substances which induce apoptosis, consists of the use of cells which express integrin-associated protein (IAP), such as myelocytes, as the cells in which apoptosis is induced.

Also claimed are apoptosis-inducers (such as antibodies) identified by the method above, which bind to IAP, and drug compositions containing them.

USE - The process is used for the simple and efficient screening of potential anticancer agents, especially for the treatment of myelocytic leukaemia.

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International Patent Class (Main): A61K-039/395
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(54) METHOD OF SCREENING APOPTOSIS INDUCING SUBSTANCES

(57) The present invention provides a method of screening substances having property of causing apoptosis, and relates to a method of screening substances having property of causing apoptosis characterized by using cells which are expressing IAP (Integrin Associated Protein), and the relates to above screening method, wherein the cells used are myeloid cells, and relates to pharmaceutical compositions containing as the active ingredient the substances obtained by the above method, and the present invention makes it possible to differentiate, identify and screen readily and highly efficiently the substances, such as antibodies and the like, that have property of causing apoptosis on myeloid cells by using cells which are expressing IAP while using specific binding reactions of the substances, and the above specific substances thus obtained can be used by virtue of their characteristics as the active ingredient of pharmaceutical compositions such as anti-cancer agents and medicines for myelocytic leukemia and the like.

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useful as an antibody recognizing antigens causing the apoptosis [it is also called self-destruction of cells, phenomenon that a nuclear chromatin DNA is digested at a nucleosome unit (so-called ladder formation) to result in the death of cells] of myeloid cells and having a function of identifying them or a function of causing apoptosis on myeloid cells. Incidentally, myeloid cells include cells other than lymphoid cells, such as neutrophils, megakaryocytes, myeloblasts, myelocytes, mast cells, macrophages, monocytes and erythroblasts, and the myeloid cells according to the present invention also mean the same as mentioned above. No monoclonal antibody having the property of causing apoptosis on myeloid cells has been known so far other than the above monoclonal antibody, and hence the above monoclonal antibodies are defined to include all monoclonal antibodies having the property of causing apoptosis on myeloid cells.

[0011] The monoclonal antibody may be prepared basically as stated below.

[0012] Namely, the above monoclonal antibody may be prepared, for example, by using splenic stromal cells derived from an animal administered rG-CSF as antigens, immunizing them according to an ordinary immunization method, cell-fusing the immunized cells according to an ordinary cell fusion method, and cloning the fused cells according to an ordinary cloning method.

[0013] As a method of preparing the above monoclonal antibody can be preferably exemplified a method comprising using CF-1 cells, splenic stromal cells of an animal administered rG-CSF established as culture cell line by the present inventors, as the antigen [Blood, Vol. 80, 1914 (1992)] , fusing plasma cells (immunocyte) of a mammal immunized with the antigen with myeloma cells of a mammal such as a mouse, cloning the obtained fused cells (hybridomas), selecting clones producing the above antibody recognizing the above cell line among them, and culturing them to recover objective antibody. However, the method is only an example, and in this case, for example, not only the above CF-1 cells but also cell lines derived from human splenic stromal cells obtained according to the case of CF-1 cells may be used as the antigens properly to prepare antibodies binding to objective human myeloid cells in the same manner as in the case of the above CF-1 cells.

[0014] In the method of preparing such monoclonal antibodies, mammals to be immunized with the above antigen are not particularly restricted; it is preferable to make selection taking into account suitability with myeloma cells to be used in cell fusion, and preferably a mouse, a rat and a hamster and the like are used.

[0015] Immunization is performed according to an ordinary method, for example, by administering splenic stromal cells such as the above CF-1 cells and the like into abdominal cavity of a mammal by injection. More specifically, it is preferable to administer one diluted with or suspended in a proper amount of PBS or isotonic sodium chloride solution to an animal several times every month. It is preferable to use splenic cells removed after the final administration of the above cells as immunocytes.

[0016] As a myeloma cell of a mammal as the other parent cell fused with the above immunocytes can be used preferably known various cells including P3(P3X63Ag8.653) [J. Immunol., 123, 1548 (1978)] , p3-U1 [Current Topics in Micro-biology and Immunology, 81, 1-7 (1978)] , NS-1 [Eur. J. Immunol., 6, 511-519 (1976)] , MPC-11 [Cell, 8, 405-415 (1976)] , Sp2/0-Ag14 [Nature, 276, 269-270 (1978)] , FO [J. Immunol. Meth., 35, 1-21 (1980)] , S194 [J. Exp. Med., 148, 313-323 (1978)] and R210 [Nature, 277, 131-133 (1979)] .

[0017] The cell fusion of the above immunocyte and a myeloma cell may be performed basically according to an ordinary method, for example, a method by Milstein et al. [Methods Enzymol., 73, 3-46 (1981)] and the like.

[0018] More specifically, the above cell fusion may be performed, for example, in an ordinary nutrition medium in the presence of a fusion-accelerating agent. As a fusion-accelerating agent, polyethylene glycol (PEG) and Sendai virus (HVJ) and the like, and furthermore, adjuvants such as dimethyl sulfoxide and the like may be added properly if required in order to enhance the fusing effect. Regarding the ratios of immunocytes and myeloma cells, the former is preferably used in an amount 1-10 times that of the latter. Examples of a medium used in the above cell fusion include a RPMI-1640 medium and a MEM medium suitable for the proliferation of the above myeloma cell and other mediums ordinarily used for the culture of this kind of cell, and in addition, supplementary serum such as fetal bovine serum (FBS) may be used together.

[0019] Cell fusion is performed by mixing prescribed amounts of the above immunocytes and myeloma cells in the above medium, adding a PEG solution preheated to about 37°C , for example, PEG with an average molecular weight of the order of 1,000-6,000 to the medium, ordinarily, at a concentration of about 30-60 % (W/V), and mixing them. Subsequently, by repeating the operations of adding proper mediums one after another, centrifuging the reaction mixture and removing the supernatants can be formed objective hybridomas.

[0020] Said hybridomas are selected by culturing in an ordinary selective medium, for example, a HAT medium (medium supplemented with hypoxanthine, aminopterin and thymidine). The culture in the HAT medium is continued for a period sufficient for cells other than objective hybridomas (non-fused cells) to die out, ordinarily for several days to several weeks. Subsequently, the screening and monocloning of the hybridomas producing the objective antibodies are performed according to ordinary limiting dilution analysis.

[0021] The prepared hybridomas producing the above monoclonal antibodies may be subcultured in an ordinary medium and stored in liquid nitrogen for a long time.

[0022] In order to collect the above monoclonal anti-

clonal antibody (BMAP-1).

Fig. 18 shows an analysis (a control according to rat IgG2a, BWV1) according to immunofluorescence.

Fig. 19 shows the binding properties of the anti-mouse MHC class I antibody to BWV1 cells according to immunofluorescence.

Fig. 20 shows an analysis (a control according to rat IgG1, BWV1) according to immunofluorescence.

Fig. 21 shows the binding properties of the BMAP-1 antibody to BWV1 cells according to immunofluorescence.

Fig. 22 shows proliferation inhibitory action to BMAP-1 cells (Jurkat cells transfected with murine IAP gene)

Fig. 23 shows an analysis of apoptosis — an action to Jurkat cells transfected with expression vector (IgG 1 1μg/ml, A: Apoptosis ratio, 6.2 %)

Fig. 24 shows an analysis of apoptosis — an action of BMAP-1 to Jurkat cells transfected with expression vector (BMAP-1 1μg/ml, A: Apoptosis ratio, 3.5 %)

Fig. 25 shows an analysis of apoptosis — an action to Jurkat cells transfected with murine IAP gene (IgG 1 1μg/ml, A: Apoptosis ratio, 3.2 %)

Fig. 26 shows an analysis of apoptosis — an action of BMAP-1 to Jurkat cells transfected with murine IAP gene (BMAP-1 1μg/ml, A: Apoptosis ratio, 25.6 %)

Explanation of Symbols

[0029]

a: DNA of the thymus of a mouse administered BMAP-1 (24 hours)
b: DNA of the bone marrow of a mouse administered BMAP-1 (24 hours)
c: DNA of the bone marrow of a mouse administered BMAP-1 (8 hours)
d: DNA of the bone marrow of a mouse administered BMAP-1 (4 hours)
e: DNA of the bone marrow of a non-treated mouse (bone marrow cells)
f: Molecular weight marker

Best Mode for Carrying Out the Invention

[0030] Next, the present invention will be described further in detail according to Reference Example and Example, but the present invention is not restricted to the Example.

Reference Example

Establishment of Splenic Stromal Cells and Their Characteristics Thereof

1) Establishment of Splenic Stromal Cells

[0031] A splenic stromal cell line was established from the primary culture of the splenic cells of a C57BL/6J mouse administered rG-CSF 100μg/kg for 5 days according to serial administration of rG-CSF. Namely, this spleen was removed after the administration of rG-CSF under germ-free conditions, cultured in a 25-cm^2 plastic flask (Corning Co.) for 6 weeks and in an Isocove's modified Dulbecco's medium (IMDM) (Boehringer-Mannheim Co.) with 10 % heat-inactivated fetal bovine serum (FBS) (Sanko Junyaku, Tokyo), 100 U/ml penicillin and 100μg/ml streptomycin in an incubator under the condition of 37 °C and 5 % CO_2, and the medium was exchanged for a fresh growth medium twice a week.

[0032] In the confluent culture, the adherent cell populations (stromal cells) were harvested from the flask by using 0.05 % trypsin plus 0.02 % EDTA (Sigma Chemical Co.) in Ca-,Mg-free PBS, and were transferred into new flasks. These passages were repeated approximately once or twice a week. In the early passages (1st through 10th passages), the split ratio of the cells was 1/4 to 1/8, and subsequently the ratio was 1/16 to 1/32. The stromal cells became homogeneous and fibroblastoid after approximately the 10th passage. At the 20th passage, these stromal cells were harvested as described above and forwarded to cell cloning by using a limiting dilution technique; cell cloning was repeated twice to establish a stromal cell line (CF-1 cell line).

[0033] Subsequently, these cells were maintained in 5 ml of IMDM supplemented with 10 % heat-inactivated FBS in a 25-cm^2 flask (Corning Co.), and subcultured once every 5 days at the split ratio of 1/32. Splenic stromal cell lines can be established from other animals than mouse; for example, human splenic stromal cell lines can be established using the same method as described above by transforming the cells with an SV-40 adenovirus vector [J. Cell. Physiol., 148, 245 (1991)].

2) Characteristics of CF-1 Cells

[0034] CF-1 cells established as a cell line as described above were examined for alkaline phosphatase, acid phosphatase, β-glucuronidase, α-naphthyl acetate esterase and oil red O using standard cytochemical techniques. CF-1 cells were also characterized by immunoenzymatic histochemistry using the following monoclonal and polyclonal antibodies: mac1 (Sero-Tec.); factor-VIII-related antigen (Dakopatts) and collagen type I, collagen type III and fibronectin (Chemicon International Inc.). Phagocytosis was tested by latex bead uptake (particle diameter 1.09 μm; Sigma).

centrifuged and recovered as pellets, then suspended in 100 µl of the hybridoma culture supernatants (about 1 × 10^6/100 µl) and reacted at 4 °C for 1 hour. After they were washed with the above buffer once, an FITC-labelled goat anti-rat IgG (FC) antibody (Chemicon) was added thereto and incubated for 1 hour. After they were washed once, they were analyzed according to flow cytometry (FACScan, Becton Dickinson).

4) Purification of Antibodies

[0047] The fused cells screened in the manner of the above 3) were cultured according to an ordinary procedure, and antibodies produced in the supernatants were separated according to an ordinary procedure, and purified.

[0048] Namely, hybridomas were recovered from wells with high antibody titers to the antigens, spread in a tissue culture plastic dish (Corning Co.), cultured under the condition of 5 % CO_2 and 37 °C , proliferated, and purified according to an ordinary procedure to obtain monoclonal antibodies GSPST-1 and BMAP-1.

[0049] Regarding GSPST-1, obtained cells were injected into the abdominal cavity of a BALB/cAJc1-nu nude mouse (8-week-old, male, Nippon Kurea). Produced ascites was recovered after 10-14 days, salted out with 33 % ammonium sulfate, and dialyzed with PBS. Regarding the BMAP-1 antibody, it was cultured in a large scale in an Iscove's modified MEM medium supplemented with 10 % FBS, and the supernatants were concentrated, salted out with 33 % ammonium sulfate, dialyzed with PBS, purified again by means of a protein A column kit (Amersham), and dialyzed with PBS. Incidentally, in the above Example was described the case in which the CF-1 cells were used as antigens for immunization; however, it is possible to establish a monoclonal antibody in the same manner also in case of using other stromal cells having potency supportive of hematopoietic stem cells.

[0050] A hybridoma producing the above monoclonal antibody BMAP-1 is a novel fused cell prepared from a Wistar Imamich rat splenic cell and a mouse myeloma cell line SP2/0-Ag14 as parent cells, and was deposited on 9 August, 1993, under the name of BMAP-1 (rat mouse hybridoma) with the accession number of FERM BP-4382, at National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology in Japan [address: 1-3, Higashi-1-chome, Tsukuba-shi, Ibaraki 305, Japan] as international depositary authority according to Budapest Treaty on the international recognition of the deposit of microorganisms for the purpose of patent procedures.

5) Properties of Antibodies

(i) Reactivity of Antibodies

(Reactivity to CF-1 Cells)

[0051] The results of examining the reactivity of the obtained monoclonal antibodies GSPST-1 and BMAP-1 to CF-1 cells according to immunofluorescence analysis are shown in Fig. 1 through Fig. 3. Here, Fig. 1 shows the results of analysis of the control in the absence of an antibody, Fig. 2 the results of analysis of the binding properties of GSPST-1 to CF-1 cells, and Fig. 3 the results of analysis of the binding properties of BMAP-1 to CF-1 cells. In the drawings, vertical axes show relative number of cells and transverse axes fluorescence intensity.

[0052] As is apparent from Fig. 1 through Fig. 3, it has been revealed that monoclonal antibodies GSPST-1 and BMAP-1 have properties binding to CF-1 cells and recognize surface antigens of CF-1 cells.

(Reactivity to Bone Marrow Cells)

[0053] Next, the results of analysis of the reactivity of GSPST-1 and BMAP-1 to normal bone marrow cells according to flow cytometry (FACScan, Becton Dickinson) are shown in Fig. 4 through Fig. 6. Here, Fig. 4 shows the results of analysis of the control in the absence of an antibody, Fig. 5 the results of analysis of the binding properties of GSPST-1 to bone marrow cells, and Fig. 6 the results of analysis of the binding properties of BMAP-1 to bone marrow cells. In the drawings, vertical axes show relative number of cells and transverse axes fluorescence intensity.

[0054] As is shown in Fig. 4 through Fig. 6, it has been revealed that GSPST-1 has not a property binding to bone marrow cells at all, and that BMAP-1 has a property binding to all bone marrow cells.

(Reactivity to Myelocytic Leukemic Cell Line (NFS-60))

[0055] The results of analysis of the the reactivity of GSPST-1 and BMAP-1 to NFS-60 cells [Proc. Natl. Acad. Sci. USA, 82, 6687-6691 (1985)] according to flow cytometry (FACScan, Becton Dickinson) are shown in Fig. 7 through Fig. 10. Here, Fig. 7 shows the results of analysis of the control in the absence of an antibody, Fig. 8 shows the results of analysis of the binding properties of GSPST-1 to NFS-60 cells, Fig. 9 shows the results of analysis of the control using rat IgG1 on the market (Zymed) and Fig. 10 shows the results of analysis of the binding properties of BMAP-1 to NFS-60 cells. In the drawings, vertical axes show relative numbers of cells and transverse axes fluorescence intensity.

[0056] As is shown in Fig. 7 through Fig. 10, it has been revealed that GSPST-1 does not react with NFS-60 cells, and that BMAP-1 has a property binding to

been reported so far as described above, and hence monoclonal antibodies having such a function are ones found by the present inventors.

[0066] It became clear by direct expression cloning that the antigen recognized by BMAP-1 is murine IAP. The action of BMAP-1 was examined employing recombinant cells transfected with murine IAP genes. That is, the action of BMAP-1 on IAP expressing cells was examined by the MTS method and the analysis of DNA fragmentation by a flow cytometry employing recombinant Jurkat cells expressing murine IAP obtainable by inducing IAP genes into Jurkat cells expressing no murine IAP by an ordinary procedure. The results are shown in Figs. 22 to 26.

[0067] The MTS method is an assay method (Promega) measuring the number of living cells, and the action of BMAP-1 on recombinant Jurkat cells was examined by this method. That is, the number of living cells was measured by the MTS method, employing a culture plate with 96 wells, by adding 1, 10, 100 ng/ml and 1, 10 μ g/ml of BMAP-1 at the final concentration and 10 μg/ml of IgG1 as a control to 1 x 10^4/well/100 μl of recombinant Jurkat cells in the presence of G418 (final concentration: 1 mg/ml) (produced by Gibco BRL) and culturing them for two days. It became clear as a result of it that, as shown in Fig. 22, the recombinant Jurkat cells are prohibited from proliferating remarkably by BMAP-1.

[0068] The analysis of the DNA fragmentation of the recombinant Jurkat cells by BMAP-1 was performed by a flow cytometry (EPICS (registered trademark) XL-MCL, produced by Coulter). That is, employing a culture plate with 6 wells, IgG1 and BMAP-1 were added at the final concentration of 1 μg/ml to 1.5 x 10^5/well/3 ml of recombinant Jurkat cells in the presence of G418 (final concentration: 1 mg/ml) (produced by Gibco BRL), and the mixture was cultured for two days and then subjected to measurement. The cells were recovered from the culture plate, and cell pellets were centrifuged at 200 x g and fixed in 2 ml of cold 70 % ethanol at 4 °C for 60 minutes. Subsequently, the cells were centrifuged and resuspended in 1 ml of PBS. To 0.5 ml of the cell sample were added 0.5 ml of RNAse (Type I-A, Sigma, St. Louis, MO, USA, 1 mg/ml in PBS) and then the mixture was mixed into 1 ml of a propidium iodide (PI, Sigma, 100 μg/ml in PBS) solution. The mixed cells were incubated at room temperature in a dark place for 15 minutes, then kept at 4 °C in a dark place and subjected to measurement by a flow cytometry.

[0069] It became clear as a result of it that, as shown in Fig. 26, in the cells transfected with murine IAP genes, apoptosis is caused by BMAP-1.

[0070] On the other hand, the above action of BMAP-1 was not observed in Jurkat cells with only an expression vector and expressing no murine IAP (Fig. 24). It became clear from these matters that the antigen recognized by the BMAP-1 antibody is identical with IAP, and that IAP has a function related to apoptosis.

[0071] According to information at present, as functions of IAP are reported an action of being bound to a β chain of $\alpha_v \beta_3$ of integrin and supporting the bond between $\alpha_v \beta_3$ and vitronectin as a ligand thereof (J. Cell Biol., 123, 485-496 (1993)), an action of causing the flow of Ca^{2+} to the endothelium in adhesion between neutrophils and the endothelium (J. Biol. Chem., 268, 19931-19934 (1993)) and an action of supporting neutrophils to pass through the endothelium (Proc. Natl. Acad. Sci. USA, 92, 3978-3982 (1995)); however, no function related to apoptosis has been reported.

[0072] Since BMAP-1 is one of monoclonal antibodies being bound to myeloid cells and causing apoptosis in myeloid cells, as described above, it becomes possible to discriminate, identify and screen substances causing apoptosis in myeloid cells by utilizing cells expressing IAP as an antigen recognized by said BMAP-1 antibody specifically.

[0073] As described above, the present inventor has made it clear that the antigen recognized by the BMAP-1 antibody is identical with IAP, and that IAP has a function related to apoptosis, and has established, according to the above information, a method of screening substances causing apoptosis in myeloid cells simply and efficiently by employing cells transfected with IAP genes or cells expressing IAP.

[0074] By utilizing the apoptosis action of the substances such as monoclonal antibody obtained by the method according to the present invention, fragments thereof and substances causing apoptosis possessing binding potency to IAP to myeloid cells, since it can be thought that the substances like said monoclonal antibody and the like can kill myelocytic leukaemia cells which are thought to express antigens thereof remarkably, the substances such as said monoclonal antibody causing apoptosis in myeloid cells and fragments thereof are useful as the effective components of medical compositions such as anticancer agents and medicines for myelocytic leukemia and the like.

[0075] The method according to the present invention has been described specifically according to Examples; though as substances causing apoptosis according to the present invention are mentioned those exemplified as specific examples and typical ones, they are not always restricted thereto; it goes without saying that all the substances possessing the same characteristics and functions prepared in the same manner are included.

Industrial Applicability

[0076] The present invention makes it possible to discriminate, identify and screen substances such as antibodies and the like causing apoptosis in myeloid cells simply and efficiently by employing cells expressing IAP and utilizing the specific binding reaction thereof. The substances possessing property of causing apoptosis

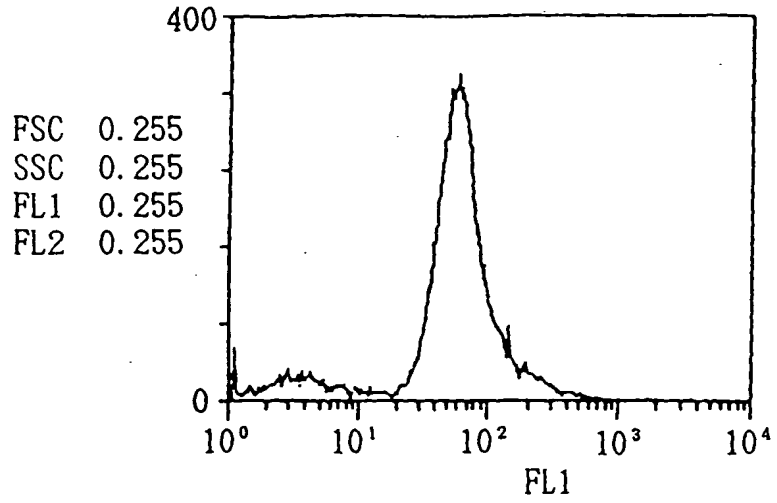
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Fig. 1

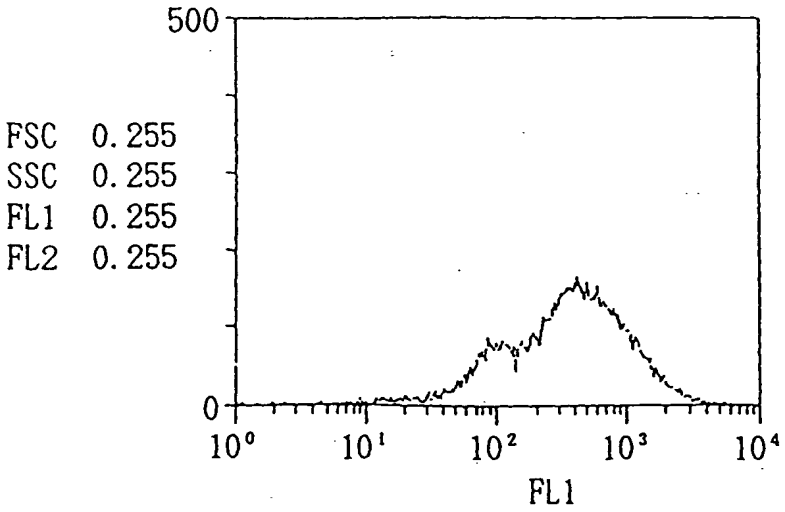
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Fig. 2

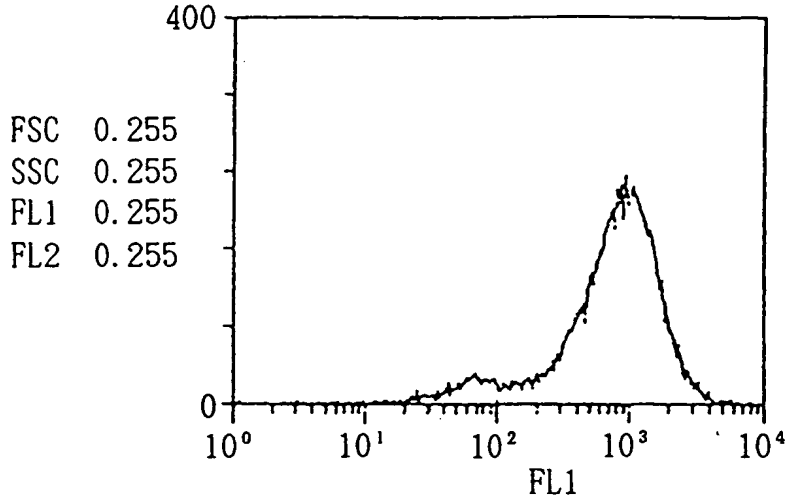
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Fig. 3

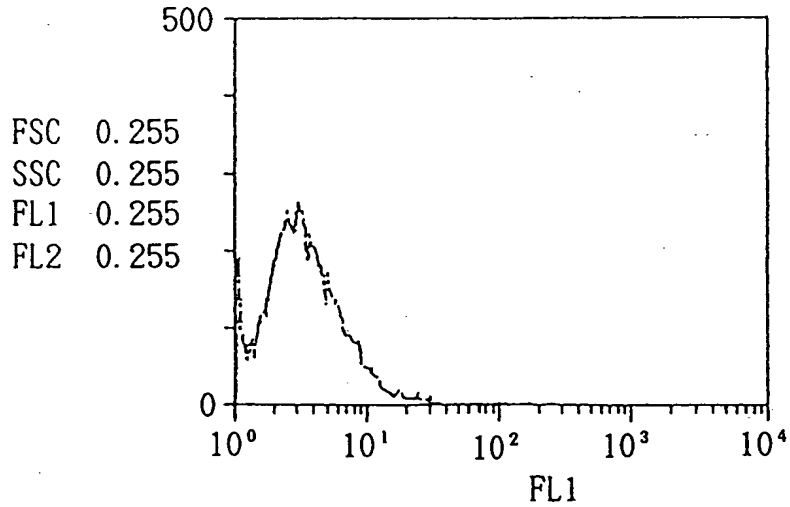
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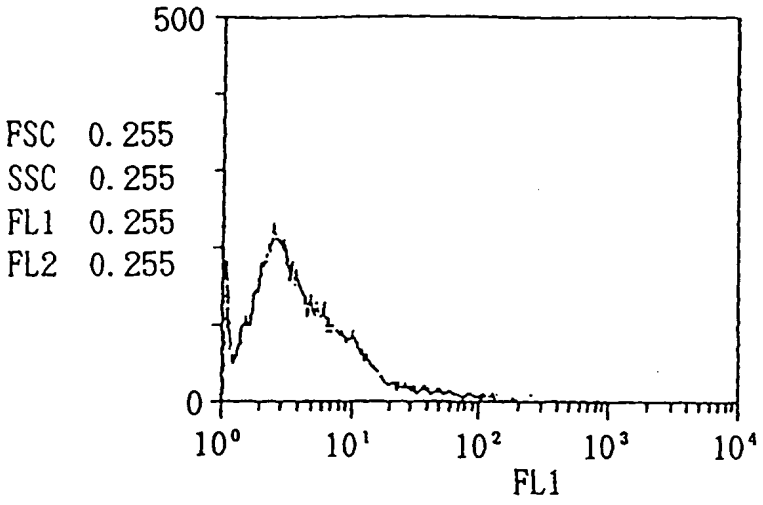
Fig. 4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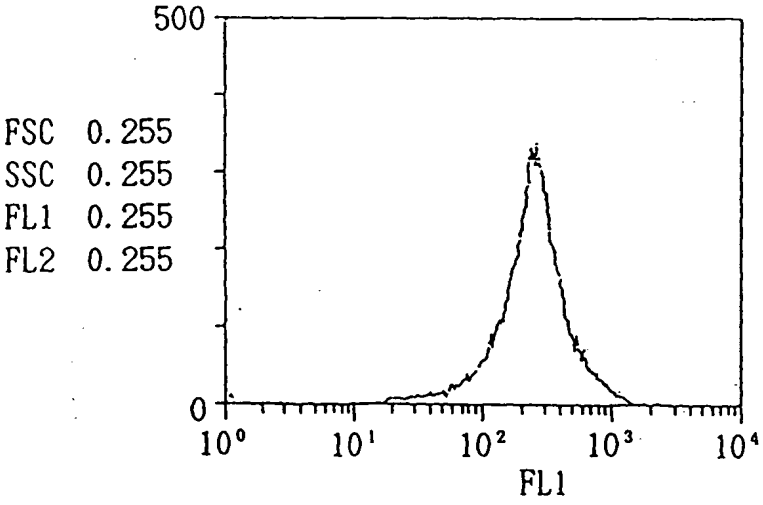
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Fig. 6

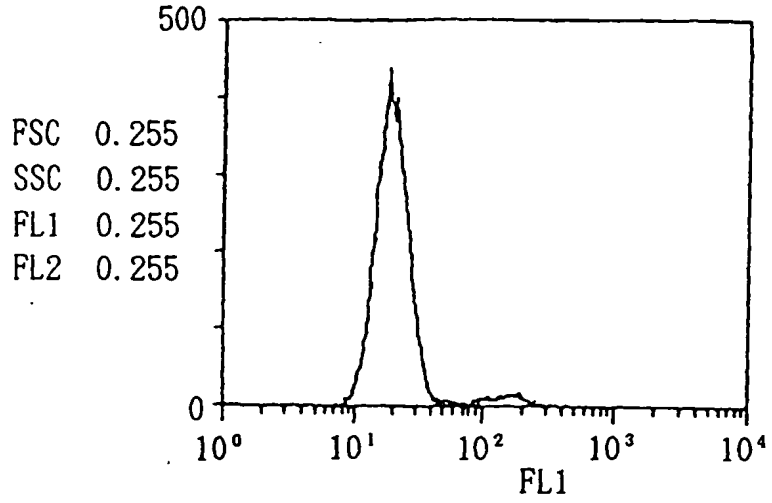
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Fig. 7

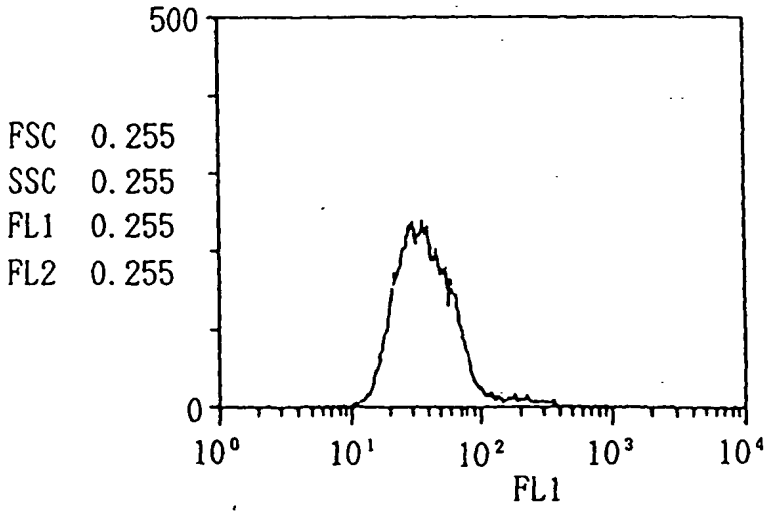
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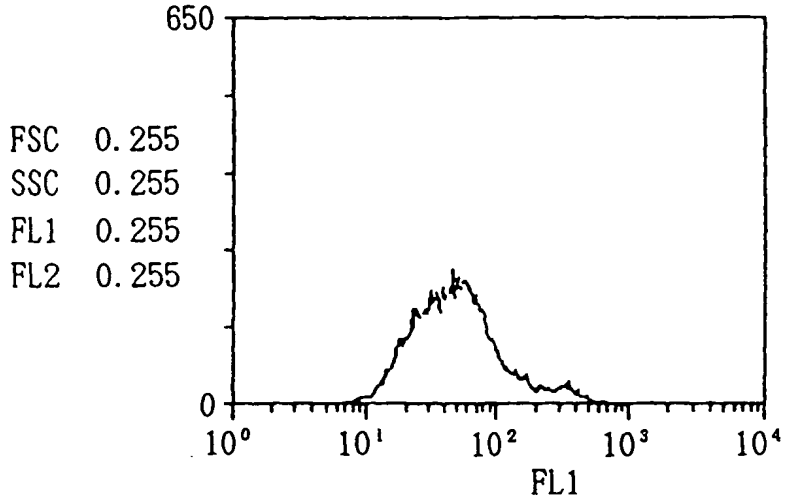
Fig. 8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SSC 0.255
FL1 0.255
FL2 0.255
0
10⁰
10¹
10²
10³
10⁴
FL1

Fig. 9

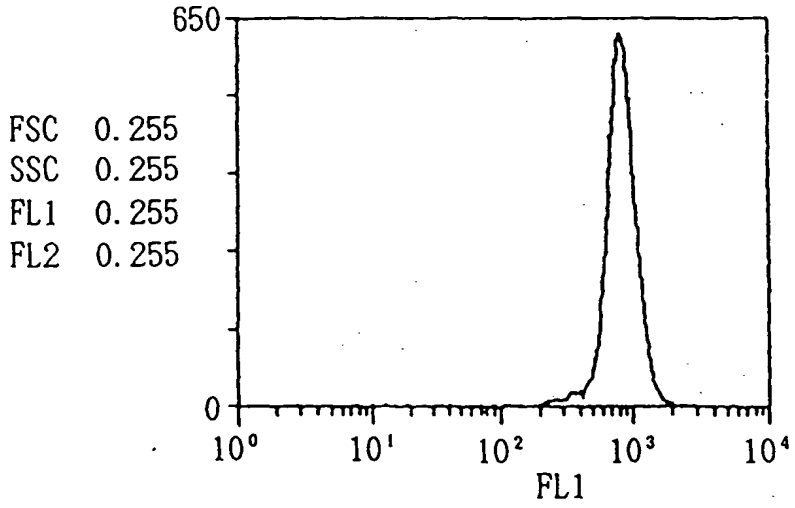
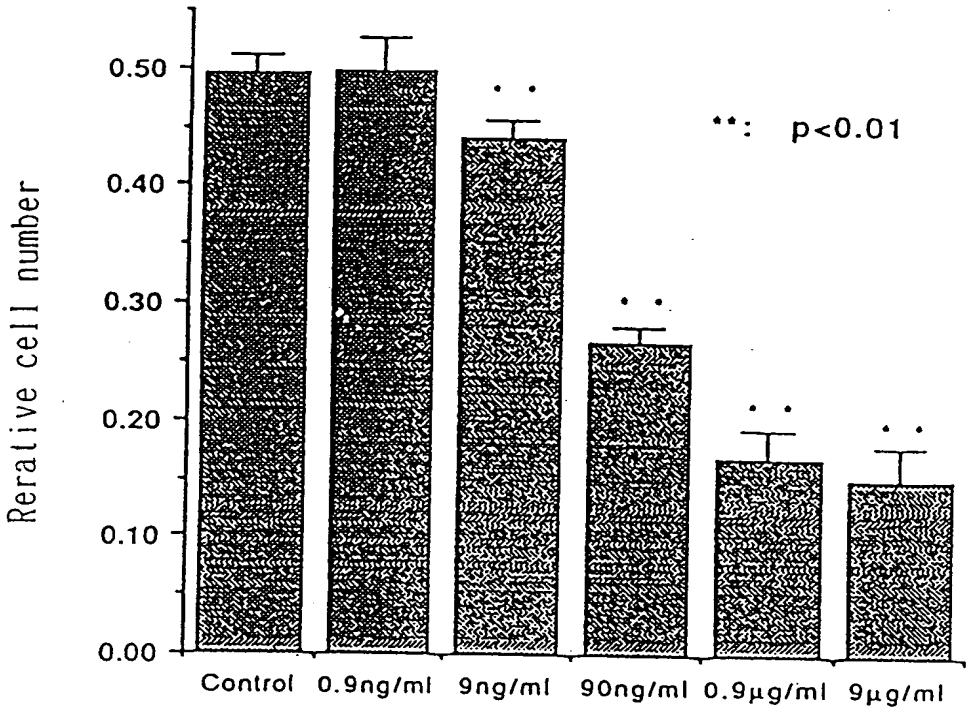

650
FSC 0.255
SSC 0.255
FL1 0.255
FL2 0.255
0
10⁰
10¹
10²
10³
10⁴
FL1

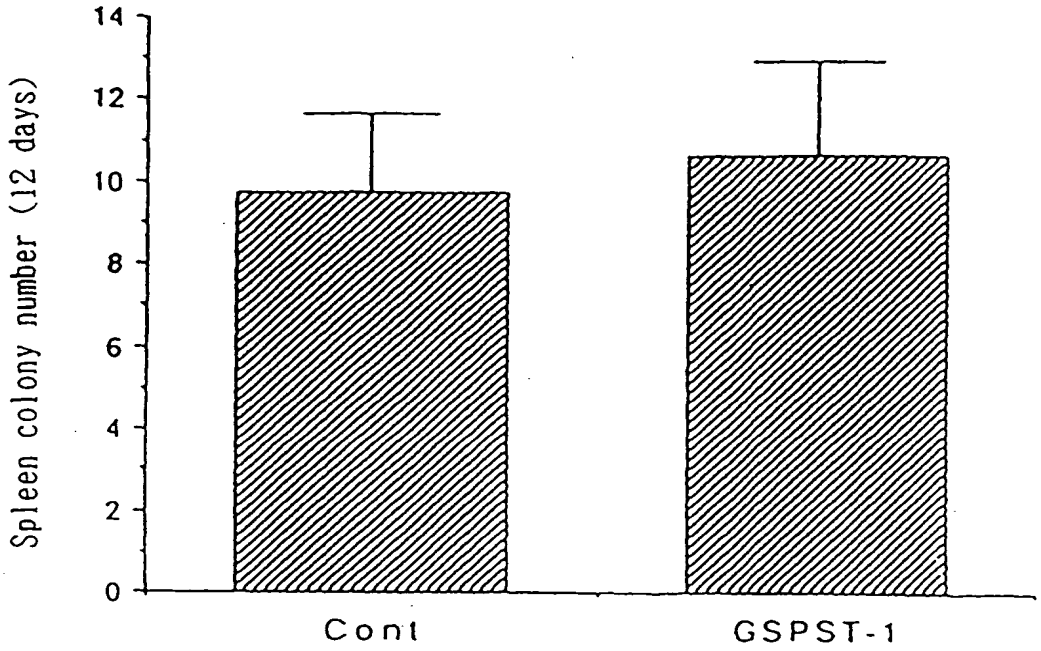
Fig. 10



Monoclonal Antibody (BMAP-1) Concentration

Assay for BMAP-1 to inhibit NFS-60 cell proliferation

Fig. 11

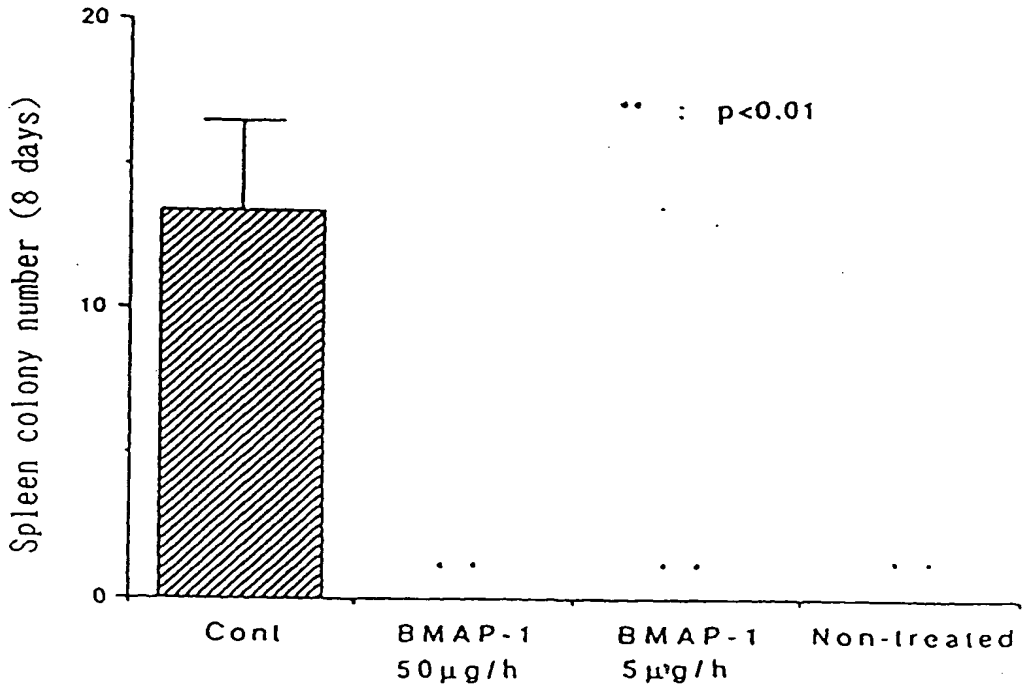


Monoclonal Antibody

Assay for GSPST-1 to inhibit

the bone marrow transplantation

Fig. 12



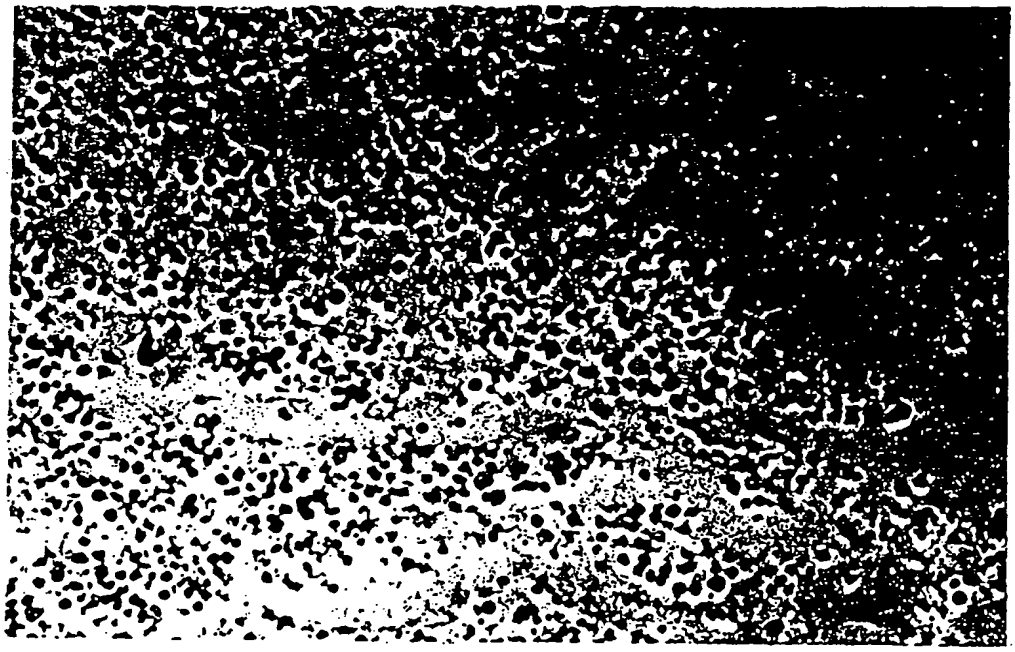
Monoclonal Antibody

Assay for BMAP-1 to inhibit

the bone marrow transplantation

Fig. 13

(1)



(2)

Fig. 14

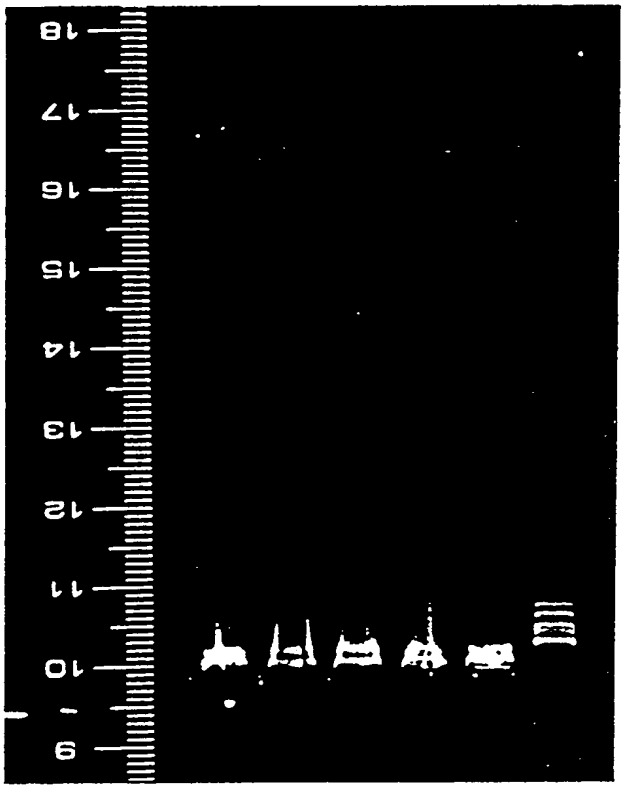
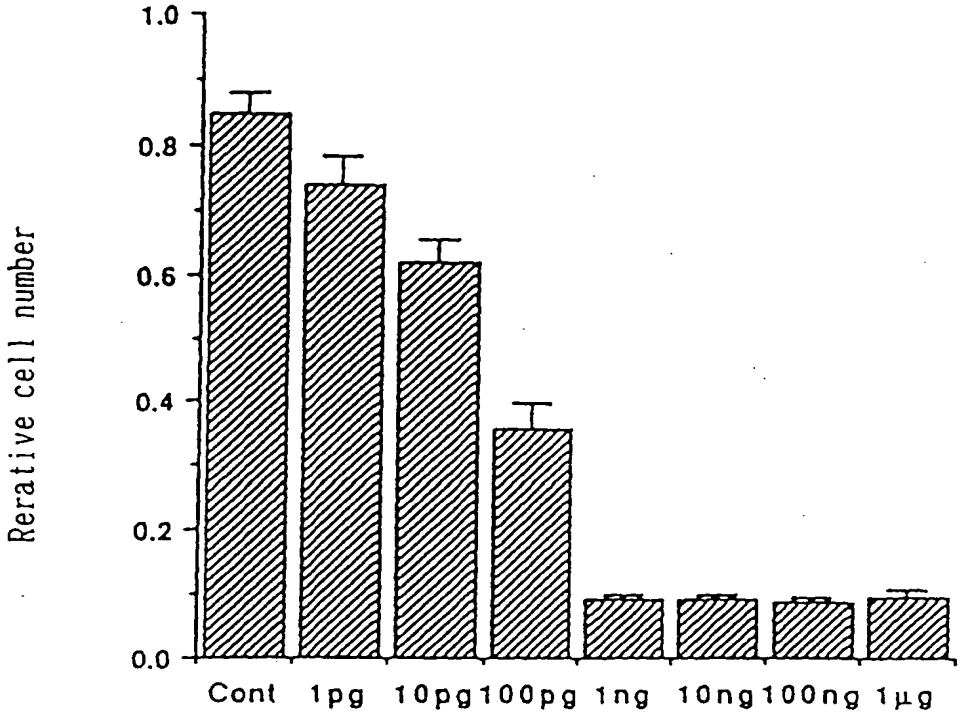
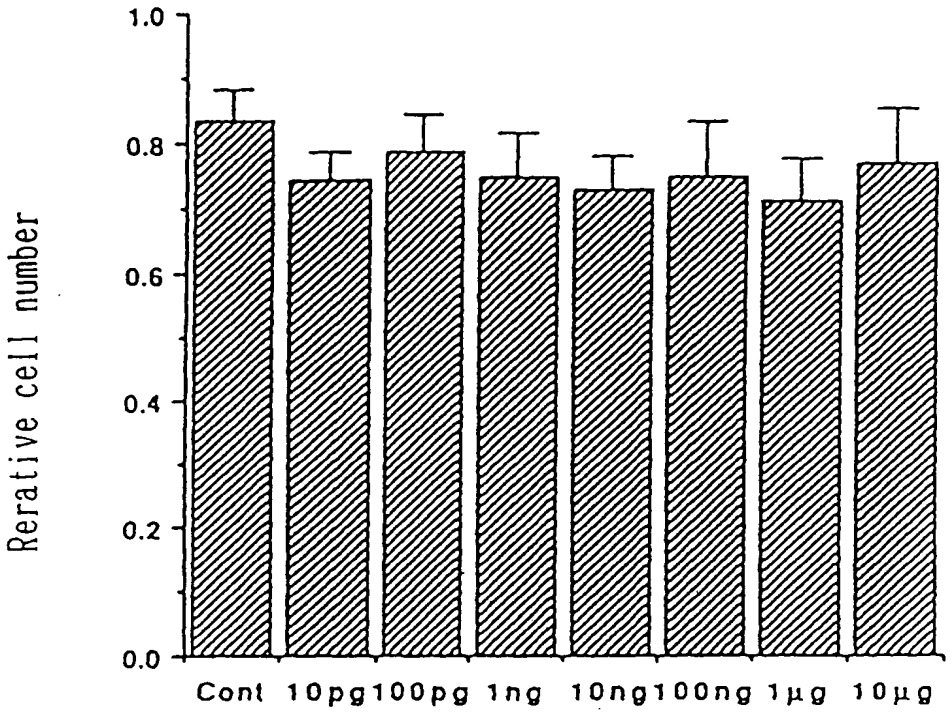


Fig. 15



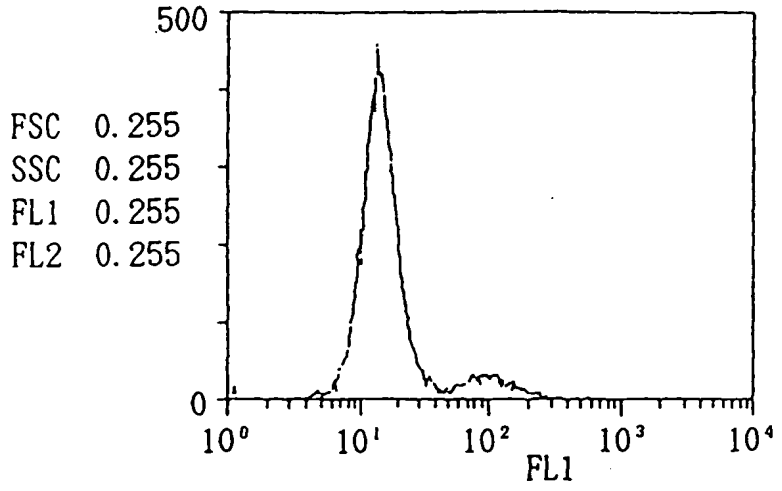
Cytotoxicity assay using L-929 cells by TNFα

Fig. 16



Cytotoxicity assay using L-929 cells by BMAP-1

Fig. 17


500
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SSC 0.255
FL1 0.255
FL2 0.255
0
10⁰
10¹
10²
10³
10⁴
FL1

Fig. 18

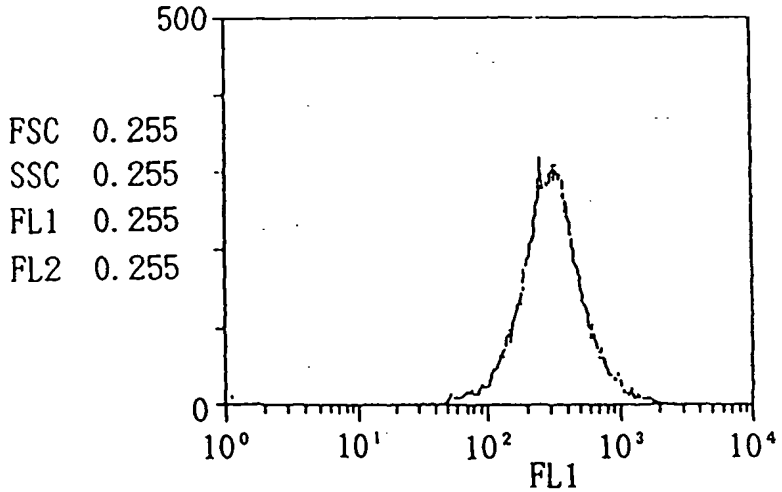

500
FSC 0.255
SSC 0.255
FL1 0.255
FL2 0.255
0
10⁰
10¹
10²
10³
10⁴
FL1

Fig. 19

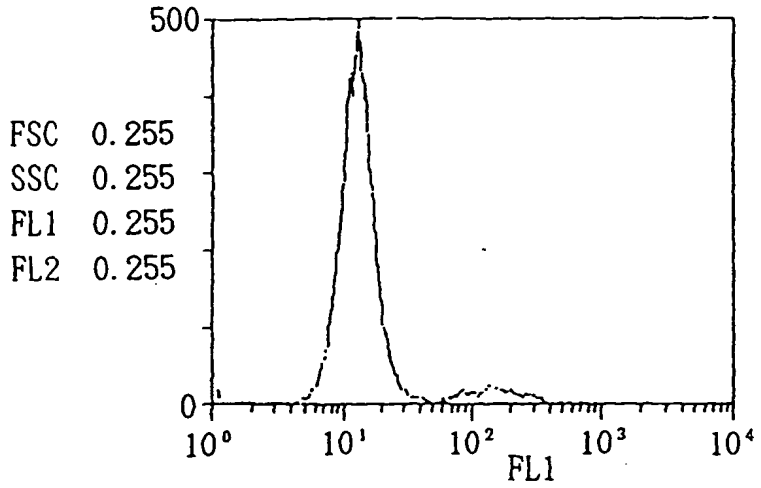

500
FSC 0.255
SSC 0.255
FL1 0.255
FL2 0.255
0
10⁰
10¹
10²
10³
10⁴
FL1

Fig. 20

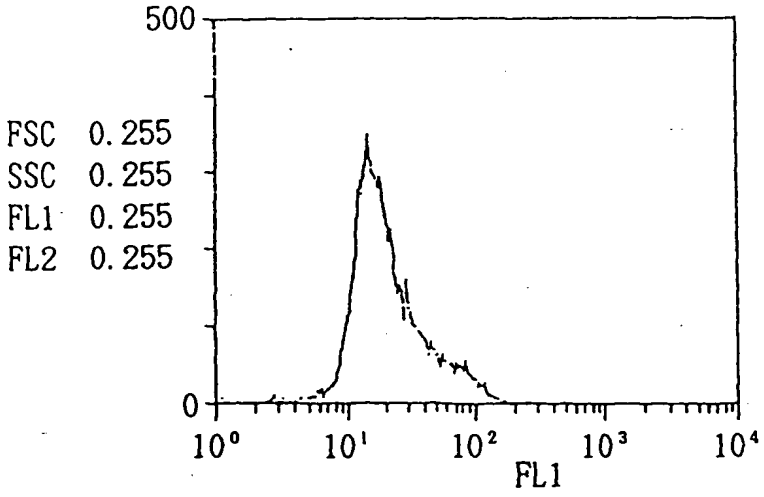
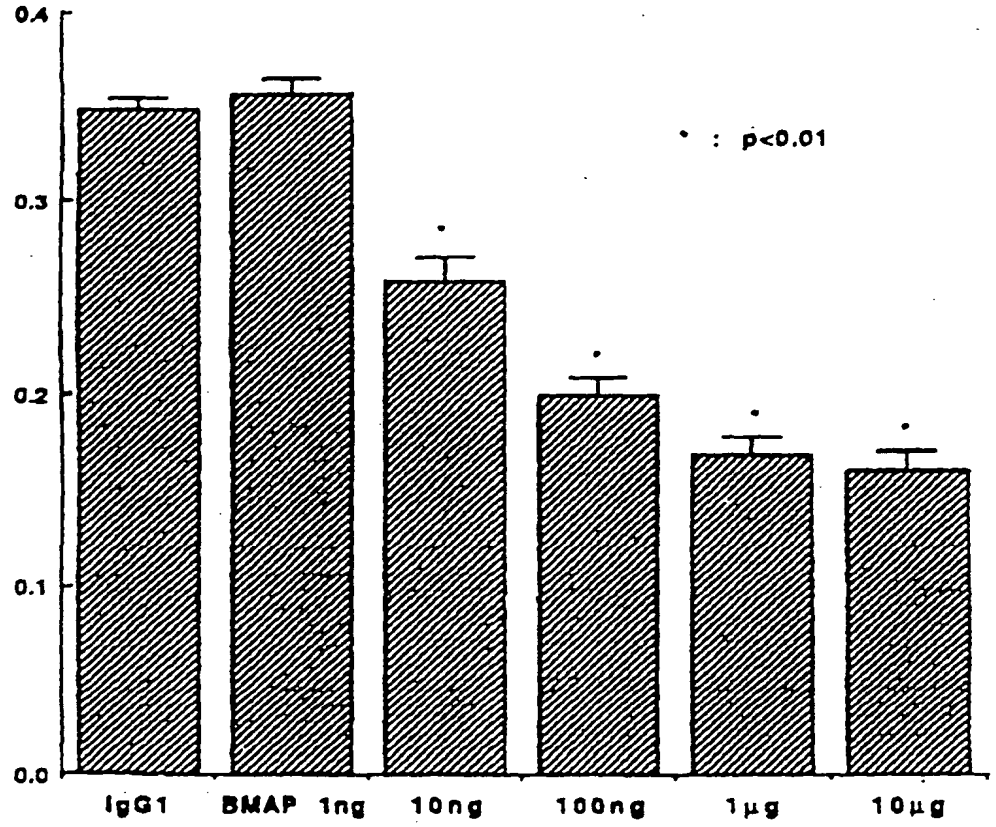

500
FSC 0.255
SSC 0.255
FL1 0.255
FL2 0.255
0
10⁰
10¹
10²
10³
10⁴
FL1

Fig. 21



BMAP-1 concentration (/ml)

Proliferation inhibitory action to BMAP-1 cells

(Jurkat cells transfected with murine IAP gene)

Fig. 22

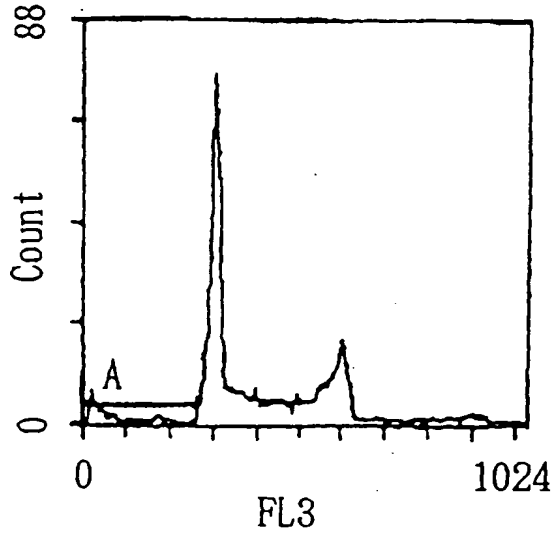


Fig. 23

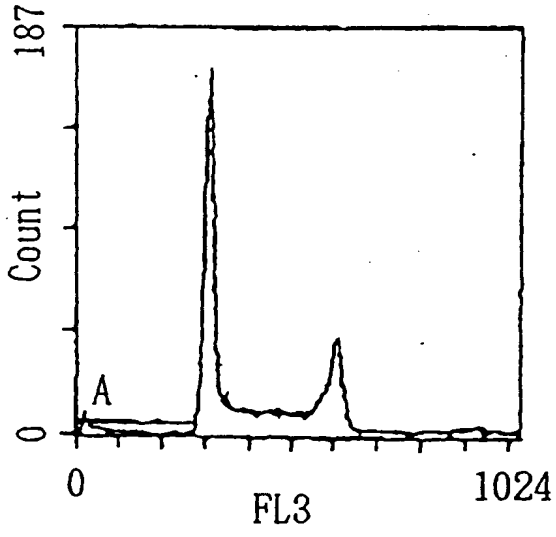


Fig. 24

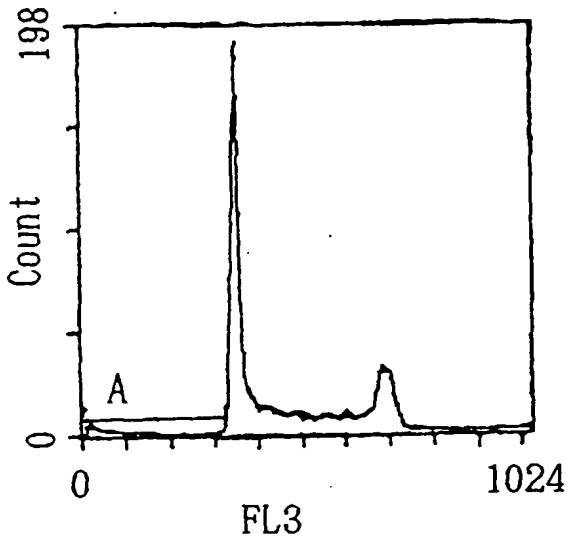


Fig. 25

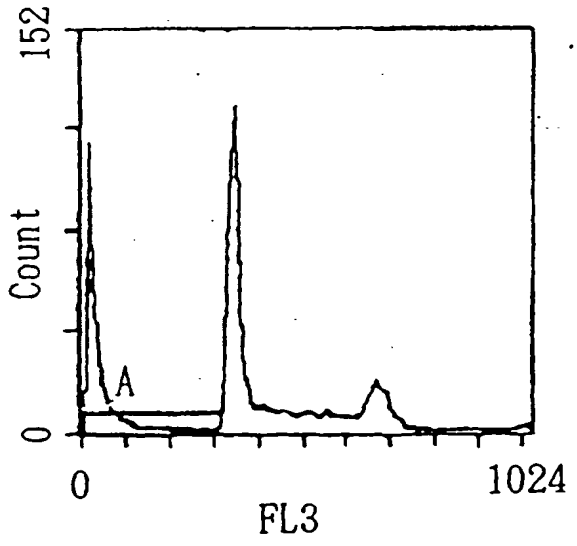


Fig. 26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP97/00702

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl^6 A61K39/395, G01N33/15//C07K16/28, C12N15/06, C12P21/08, (C12P21/08, C12R1:91)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int. Cl^6 A61K39/395, G01N33/15//C07K16/28, C12N15/06, C12P21/08, (C12P21/08, C12R1:91)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, 95/06748, A1 (Chugai Pharmaceutical Co., Ltd.), March 9, 1995 (09. 03. 95) & EP, 721015, A	1 - 7
Y	ROSALES, Carlos et al., "EXPRESSION OF THE 50-kDa INTEGRIN-ASSOCIATED PROTEIN ON THE MYELOID CELLS AND ERYTHROCYTES", THE JOURNAL OF IMMUNOLOGY, 1992, Vol. 149, No. 8, pp. 2759-2764	1 - 11
Y	BROWN, Eric et al., "Integrin-associated Protein: A 50-kD Plasma Membrane Antigen Physically and Functionally Associated with Integrins", The Journal of Cell Biology, 1990, Vol. 111, No. 6, pp. 2785-2794	1 - 11
Y	DEDHAR, Shoukat, "Integrin mediated signal transduction in oncogenesis: An overview", Cancer and Metastasis Reviews, 1995, Vol. 14, pp. 165-172	1 - 11

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* Special categories of cited documents:
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"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
May 16, 1997 (16. 05. 97)	May 27, 1997 (27. 05. 97)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)